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THE GRADIENT RP-HPLC-DAD METHOD FOR THE QUANTITATIVE DETERMINATION OF METRIBUZIN HERBICIDE IN SOME FOOD STUFFS RESIDUE

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ABSTRACT

A simple, sensitive and rapid solid-phase extraction (SPE) supported Reverse Phase – High Performance liquid Chromatography – Diode Array Detector (RP-HPLC-DAD) method has been presented for the routine determination of metribuzin in some common food stuffs and water samples. Equivalent to 0.1% phosphoric acid and methanol: water (20:80 v/v) as mobile phase and flow rate was set at 0.8 mL/minute. C-18 PRP, YDAC-218 HPLC column and DAD detector were selected on the basis of physical and structural properties of metribuzin. The LOD and LOQ values have been found to be 1 and 10 µgL-1 respectively. The developed method shows satisfactory spiked recoveries of the metribuzin from some food stuffs viz. soy bean, sugarcane, tomato, potato and water samples ranging from 81.96 to 94.20% with relative standard deviations below 3 %. The minimum consumption of solvent (10 mL ethyl acetate) in SPE for residual workup in 5 minutes, with subsequent 20 minutes RP-HPLC analysis makes the proposed method simple and rapid having promise as an excellent economical alternative for the quantitative determination of metribuzin in food stuffs.

KEYWORDS: Metribuzin, Solid-Phase Extraction, RP-HPLC-DAD & Food Stuffs

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Original Article

INTRODUCTION

Modern agriculture depends upon the huge use of herbicides in order to control the weeds that compete with crops and reduces the food productivity. Triazines have been used as a selective herbicide in agriculture as a pre-emergence or post emergence herbicide around the world for more than 50 years. Metribuzin (4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) (Figure 1) is also a triazine herbicide and acts by inhibiting photosynthesis by disrupting photosystem II [1]. It is a selective and systemic herbicide used for control of many grasses and broad – leaved weeds in soya beans, sugarcane, tomato, potato and many other at 0.07-1.05 Kg a.i./ha [2,3]. It is highly soluble in water (1.05 g/L) and weak adsorption in low organic sandy soil, sorption coefficient vary from 0.56 in sandy loam to31.7 in a soil containing 60% organic matter[4,5] consequently found to contaminate surface and ground water. According to U.S. Environmental Agency Health Advisory Report Metribuzin level for drinking water is 175 µg L^{-1} and Metribuzin concentration in ground water were reported in the range of 0.60-6.80 µg L^{-1} [6-8]. As per its environmental fate, Metribuzin undergoes degradation through various processes like photochemical, chemical and biochemical. Metribuzin shows different degradation rate in different conditions as; in natural pondwater, it undergoes photolysis having half- life of <7 days, biodegradation in soil having half-lives from 1.5-4 months and photodecomposition on the soil surface proceeds with the half-life of

14-25 days [9-12].

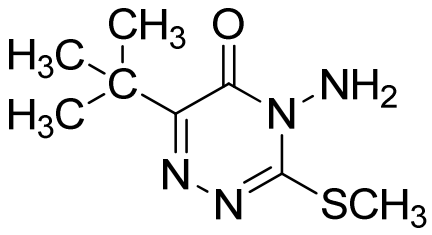

Figure 1: Structure of Metribuzin

Metribuzin shows adverse effects on human and animals. The presence of this compound in food stuffs and in drinking water is of serious health concerns. Several animal studies are available on metribuzin showing developmental effects i.e. maternal toxic effects are accompanied by toxic effects on the fetus[13]. The effects include the reduction in maternal body weight gain, consumption of food and fetal mortality. The wide agricultural use of his herbicide and associated health hazards the analysis of this herbicide on food stuffs and in water samples to access their environmental toxicity and health hazards is important and analysis of herbicide in their commercial formulation is equally important to ensure the quality of the marketed products for effective herbicide control and also to obtain reliable residue data. The presently available method for the determination of the metribuzin residues involve spectrophotometric [15], electrochemical [16], micellarelectrokinetic chromatography [17], gas chromatography (GC) [19,20] and high performance liquid chromatography (HPLC) [21-24]. Methods based on later techniques find wide favour for its analysis. However, there is a scope for such simple and sensitive methods for analysis of this herbicide. HPLC is preferred over GC methods as the former coupled with SPME increase the sensitivity in the determination of metribuzin[25-27]. Even these methods are very costly and time consuming in comparison to methods employing UV-visible and DAD detectors. As a uv-visible detector has only one sample receiving section, a DAD detector has multiple photodiode arrays to obtain the information over a wide range of wavelength at one time, which is the merit of DAD detector and thus makes the method simple, rapid and economical. In this report one such "Gradient RP-HPLC-DAD Method for the Quantitative Determination of Metribuzin herbicide in residue on some Food Stuffs**"** has been developed for the routine determination of this herbicide in food stuffs and water samples. Liquid-Liquid extraction is widely used for extraction of herbicide and its clean-up for matrix interferences removal. We have also proposed SPE for the purpose which involves minimum consumption of solvent and is a good alternative to conventional extraction procedures reported. A cost effective SPE (silica based, 60 mesh, 1.6 cm i.d. homogenous glass column) with ethyl acetate elution at room temperature has been contrive for residual workup. The extraction is complete in 5 minutes followed by 20 minutes rapid and selective HPLC method.

REAGENTS AND MATERIALS

Standard Metribuzin (Product No-36165, GC purity, 99.0%) was purchased from Sigma-Aldrich, Bangalore, India. Ethyl acetate and Methanol (HPLC Grade; Merck, Germany) were used as such. AR grade chemicals were used in the analysis.

INSTRUMENTATION AND METHOD

An Agilent 1200 series HPLC system (Germany) with Chemstation software, 6.0 version using DAD detector was used to perform RP-HPLC analysis. The HPLC chromatographic Column C-18 PRP VYDAC-218TP (length 150X 5µm,

i.d. 2.1 mm and column temperature 25^0c). The injection volume was 10µL.

Mobile Phase: A: 0.1% Phosphoric acid; B: Methanol: Water (20:80 v/v Gradient) with a constant flow of 0.8mL/minute was optimised and set in the gradient reverse phase mode.

Table 1: HPLC Gradient

Time (minute)	% B
0	30
5	70
8	100
15	100
20	30

Column Preparation for SPE

Glass column of 30 cm length and 1.6cm diameter was used. Firstly silica 15g (Silica gel 60-120 mesh) for column chromatography (Sisco Research Laboratory Pvt. Ltd., Mumbai) was taken in beaker added 20 mL of ethyl acetate and the column was filled with this slurry slowly so that silica spread homogeneously in the column [18].

Preparation of Standard Calibration Curve

For the preparation of RP-HPLC calibration curve standard 100 ppm solution of metribuzin was prepared in methanol and then diluted up to 10^{-1}µg $L^{-1}$with the same solvent. Calibration curve was prepared for quantitative determination using the solutions prepared according to Table 2. RP-HPLC analysis was performed in a 20 minute run time method. Following HPCC gradient with %of methanol: water (20:80 v/v Gradient); 30%(0 minute), 70% (5 minute), 100% (8 minute), 100% (15 minute) and 30% (20 minute). 10 µL metribuzin sample was injected in each run and peak area was measured from DAD detector. Calibration curve was constructed by plotting peak area versus concentration of standard atrazine solutions (Figure 2).

Table 2: RP-HPLC Quantization Limits for Standard Metribuzin Solutions

Dilution No.	Concentration (µg L^{-1})	Retention Time (Min.)	Area	HPLC Limits	S/N Ratio
1	10^5	15.2	35432		48
2	10^4	15.2	30067		38.2
3	10^3	15.2	23865		20.7
4	10^2	15.2	16764		15.8
5	10	15.2	14022	LOQ	11.4
6	1	15.2	6741	LOD	4.1
7	10^{-1}	15.2	2915		2.9

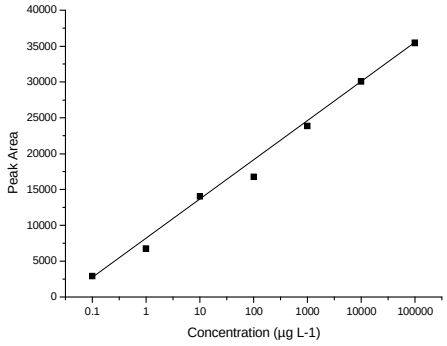

Figure 2: Standard Calibration Curve of Metribuzin

Formulation Analysis

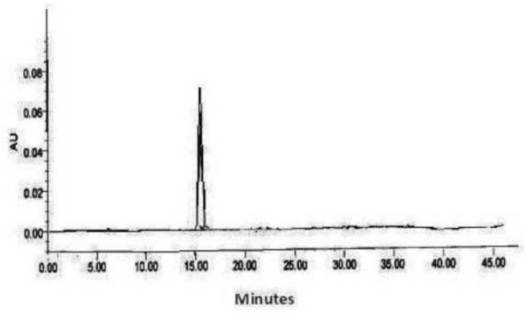

Figure 3: Typical RP-HPLC Chromatogram of Commercial Metribuzin Formulation

A Wettable Powder (WP) formulation of Metribuzin purchased from Bayer crop science India containing 70% active metribuzin ingredient was used. Standard 100ppm solution was prepared in methanol and then solution of 100µg $L^{-1}$was prepared by dilution with same solvent. Aliquots of 100µg L^{-1}solution were taken and diluted to 5 ml with methanol and processed for RP-HPLC analysis in the same manner as described above (Figure 3). The recovery of the active ingredient from the formulation has been found in the range 68.9-69.93% with relative standard deviation in the range 3%. The maker's specification has also been established by a reference method [14].

Recovery Experiment

Standard solution of 200 x 10^3µg $L^{-1}$of metribuzin was prepared in methanol and then solution of 10µg $L^{-1}$was prepared by dilution with the same solvent. Aliquots of this solution were added to 5g portion of each grain material. The samples were mixed thoroughly and extracted with 10 mL of ethyl acetate. The extract was purified by passing through the silica column extractor at a flow rate of 0.7mL/min. The eluate collected was dried with nitrogen gas drier and the remainder was dissolved in10 ml methanol and analysed with RP-HPLC-DAD method developed above.

RESULTS AND DISCUSSIONS

In the present work a new simple, sensitive and rapid RP-HPLC-DAD method for the quantitative determination of metribuzin in some food stuffs has been developed. An Agilent 1200 series HPLC system (Germany) with Chemstation software, 6.0 version using DAD detector was employed. The selection of HPLC chromatographic Column C-18 PRP VYDAC-218TP (length 150X 5µm, i.d. 2.1 mm and column temperature 25^0c) and Diode array Detector (DAD)was based on the structure with hydrocarbon chain present in the metribuzin. Correlation coefficient 0.6782 was calculated from Regression Equation y = 325.170x + 2914.967. Though MS/MS detectors are more quantitative and sensitive over DAD, but metribuzin gives a good UV-visible absorption at 293 nm and comparative low cost of DAD over mass detector expertise the preferred use of DAD for excellent linearity limits (LOL). Run time of 20 minutes with gradient flow of mobile phase with 0.8 mL/minute permits the determination of herbicide. Estimated LOD and LOQ values have been found 10and 10µg L^{-1}respectively.

A cost effective SPE column (Silica based, 60mesh, 1.2 cm i.d homogeneous glass column) with ethyl acetate elution at room temperature has been contrive for pre-concentration workup. The samples were extracted with ethyl acetate then purified with a silica SPE column, and finally, detected by RP-HPLC-DAD. The silica gel adsorbents are widely used and one of the best adsorbents which are cheaper and used in column chromatography. The reason for selecting silica gel

in chromatography is that it remains neutral and does not interact with metribuzin residues that are passed through it. It exists in its own stable structure throughout the process. Importantly, it can be recycled or reused many times by heating to a specific temperature (about 150 ^{0}C), when it releases all the substances absorbed by it and thus lower the cost of purification considerably.

All data were subject to strict quality control procedures, including the analysis of procedural blanks and spiked samples with each set of samples analysed. Metribuzin was not detected in the procedural blanks and method performance was assessed using spiked food samples. Recovery experiments of four fortified concentrations were carried out on grain, grass, fruit and vegetables i.e. soya bean, sugarcane, tomato and potato respectively samples which did not contain the target compound. The data of recovery and precision experiments are shown in Table 3 and chromatograms are represented in Figure 4. The method was shown to have good precision and high recoveries. Average recovery and maximum standard deviation of the analytical method applied were 81.96 to 94.20% of spiked amount and 2.68% respectively. The method has successfully applied to the analysis of metribuzin in its commercial formulation and residues in some food stuffs. The former is essential not only to ensure the quality of marketed products but also to get reliable residue data.

Table 3: Recovery of Metribuzin from Spiked Grains, Grass, Fruit and Vegetable Matrices

Metribuzin in µg L^{-1}	Recovery (%)[a]			
	Soy Bean	Sugarcane	Tomato	Potato
10	90.51 ± 1.59	87.12 ± 1.30	81.96 ± 2.39	89.13 ± 2.20
20	91.92 ± 0.72	89.44 ± 2.68	88.50 ± 1.65	89.38 ± 1.39
30	94.20 ± 2.31	90.00 ± 0.88	85.26 ± 2.14	90.10 ± 2.43
40	93.44 ± 2.38	88.18 ± 1.56	87.56 ± 2.50	91.60 ± 1.61

[a]**Values are mean ±standard deviation for five determination**

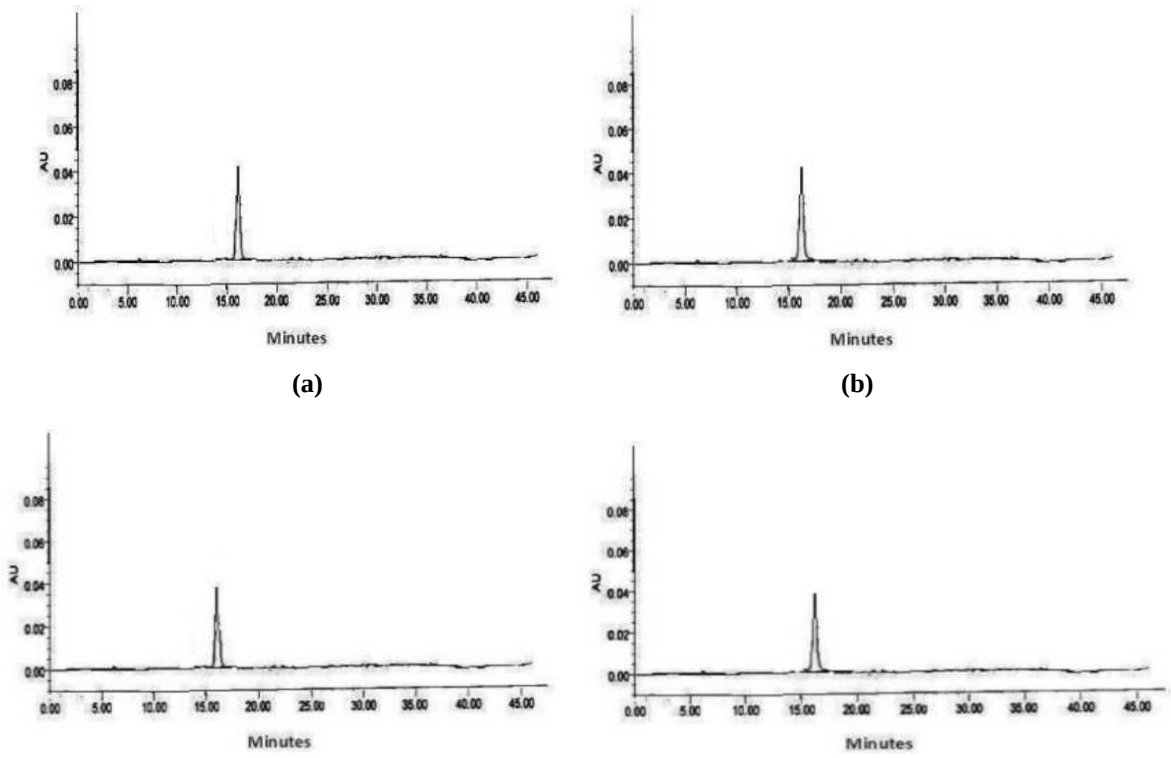

Figure 4: RP-HPLC Chromatograms for Soybean (a), Sugarcane (b), Tomato (c) and Potato (d) Matrices Respectively

CONCLUSIONS

In this paper, a simple and rapid method was developed and described for the routine screening of determination of metribuzin herbicide residues in some food stuffs. The samples were extracted with ethyl acetate and purified with a cost effective SPE Silica based glass column. Ethyl acetate elution at room temperature in five minutes, with subsequent 20 minutes RP-HPLC-DAD detection makes the proposed method fast, simple and sensitive and could be potentially extended to other matrices. The selectivity, linear range, recovery, precision, and limit of quantification were all evaluated and verified. LOD is 1µg L^{-1} and keeps pace with the advances of international technology.

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LIST OF FIGURE CAPTIONS

LIST OF TABLE CAPTIONS